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SENSITIVITY AND MUTAGENIC EFFECTS OF ETHYL METHANE SULPHONATE ON THE GROWTH OF *CROSSANDRA INFUNDIBULIFORMIS* L. NEES

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ABSTRACT

The experiment aims to examine the effects of chemical mutagen *viz.,* Ethyl Methane Sulphonate (20, 30, 40, 50 and 60 mM) on various characters *viz.,* seed germination, seedling survival and growth parameters. The results indicate that all the treatments affected the growth of the plants but sensitivity of crossandra to different concentrations was different. Sensitivity to EMS was determined by various measurements on the vM_1 generation. EMS treatments recorded less than 50 per cent germination and survival in all the concentrations. Higher doses were found to be detrimental for germination and growth parameters. In general, the variance was increased for all the characters under study in the treated populations compared with control suggesting an increase in genetic variability. However, the concentration 30 mM has recorded the higher internodal length, no. of leaves, length and breadth of leaf, no. of branches and length of branch than control.

KEYWORDS: Crossandra, Sensitivity, Mutation, Germination, Survival

INTRODUCTION

Crossandra infundibuliformis (L.) Nees is commonly called as Fire Cracker flower belonging to Acanthaceae family is native to Asia, South America, South Africa and Madagascar. The genus consists of 20-25 species and commercially cultivated species is *Crossandra infundibuliformis.* It is cultivated mainly for loose flower purpose. Being an important commercial loose flower crop in south India there is limited variability in crossandra. Efforts were initiated through to induce variability through EMS and their effects on growth parameters were investigated. The application of mutagenesis has vast potential for increasing the available genetic variation. Induction of mutations based on physical/chemical mutagens is one of the major breeding approaches for plant improvement. Therefore, induced mutagenesis through irradiation or chemical treatment has become a very important method for plant breeding, including flower breeding. Particularly EMS has been successfully used on chrysanthemum, yielding a frequency of 5.2% mutants. Though there are number of chemical mutagens, for practical purpose of induction of mutation, EMS is really functional. A wide range of variations in petal color (pink-salmon, light-pink, bronze, white, yellow and salmon color) have been recorded (Jain, 2010). By the year 2000, over 2200 mutant varieties of ornamental plants had been released worldwide (IAEA, 2005), including 175 plant species with induced mutant varieties (Maluszynski *et al.,* 2000). The basic requirement

for an effective use of mutation induction is to analyze the efficacy of mutagen and the effective does of inducing mutation. These characters were reported to be influenced due to mutagenic effects causing changes in the DNA and damage to metabolic systems (Mickaelson, 1968). Information on the differential sensitivity of the genotype to different mutagenic treatments is not available for most of the commercial loose flower crops and in particular of crossandra. Hence, the present investigation was carried out to study the effects of ethyl methane sulphonate on seed germination, seedling survival and growth parameters.

MATERIALS AND METHODS

The experiment was conducted at Horticultural College and Research Institute, Periyakulam located at a north latitude of 10^0 1' and 81^0 1' east longitude during 2013-14 to evaluate the effect of different concentrations of EMS on survival and performance of crossandra. Local crossandra type was used for the study. The experiment was laid out in randomized block design with four replications. The treatment details *viz*., Control (Wet) – Untreated seeds, 20, 30, 40, 50 and 60 mM. Fresh, healthy seeds of crossandra were soaked in water for $1^1{}_{/2}$ hours to soften the seed coat. The duration of pre-treatment with water was determined by a preliminary blank experiment. It was observed that there was about 10-15 per cent radicle emergence 6 hours after soaking in water while there was no radicle emergence when the seeds were soaked for 3 hours. An initial pre-soaking period of $1^1{}_{/2}$ hours in water was therefore adopted for treatment with mutagens.

The required concentration of EMS solution was prepared in double distilled water (pH 7.0). The treatment was performed at the room temperature of 25± 1° C from 6.00 a.m. to 12.00 noon with intermittent shaking during the treatment period. After the treatment with chemical, the seeds were thoroughly washed in running tap water for 30 minutes and were spread gently over a blotting paper to remove the excess moisture on the seed coat. To ensure uniform absorption of the mutagen, the volume of the mutagen solution was maintained at a proportion of five times to that of seed volume (Raut, 1969). After imposing treatments, seeds were sown in protrays containing sterilized cocopeat. Lethal Dose was determined by measuring the seed germination, seedling height, survival percentage and emergence of the M_1 generation under field conditions. When the seedlings attained four leaf stages, they were transplanted in the main field. Data's were recorded on germination, survival and growth characters in the M_1 generation. The data of the field observations were analyzed using 'F' test for significance following the methods described by Panse and Sukhatme (1964). The values recorded in percentage were transformed in to angular values prior to analysis wherever necessary.

RESULTS

All the concentrations of EMS affected the germination of seeds considerably. Among the treatments EMS at 30 mM has recorded the higher germination percentage whereas at 60 mM has recorded the lowest. The percentage of germination of seeds ranged from 35.65 in 30 mM to 31.79 in 60 mM whereas it was 55.50 in the control. The LD_{50} value for the germination was observed to be below 50 per cent. There was no consistent trend in the percentage of survival of seedlings as influenced by different concentrations of EMS. The percentage of reduction was 7.15, 47.78, 45.43, 49.10 and 49.47 at 20, 30, 40, 50 and 60 mM concentrations respectively over control (Table 1). However, there was a significant difference between the treatments. The relative mutagenic sensitivity of crossandra was measured in the M_1 generation of direct treatment by recording germination, survival and growth parameters. The per cent germination and survival decreased with increased in the concentration of EMS. The LD_{50} for germination and survival in EMS was even the lower concentration recorded less than 50 per cent survival in M_1 generation.

By using seedling height at transplanting on the 45th day as a criterion for measuring the sensitivity perceptible differences were observed among the mutagen. The seedling height was ranged from 12.37 to 9.76 cm at 20 and 60 mM. The data on seedling height have indicated growth reduction with increase in the concentrations of EMS in M_1 generation. The plant height was increased from 76.98 cm in control to 84.32 cm at 20 mM and 82.57 at 30 mM treatments. However, the height was decreased at higher concentrations of 50 mM and 60 mM. EMS at higher concentrations has produced more number of leaves. The no. of leaves varied from 218.59 at 60 mM to 209.57 at 20 mM over 195.43 leaves in control. The internodal length was 3.74 and 4.36 cm at 20 and 30 mM concentrations while in control it was 3.66 cm. The length of leaf showed 10.78 and 10.54 per cent increase at 30 and 40 mM concentrations of EMS over 9.75 at control. The breadth of leaf at 20, 30 and 40 mM concentrations increased and accounted for 4.67, 5.26 and 4.45 respectively as compared to 4.35 at control. The maximum number of branches 28.29 was recorded at 30 mM whereas the minimum was 20.93 observed at 60 mM than 16.81 at control. The length of the branch was higher at 30 and 40 mM concentrations accounting for 29.23 and 26.56 over control. The length was almost equal to the control 25.58 at 20 mM and reduced 20.58 at 60mM (Table 2).

DISCUSSIONS

In the study seeds of *crossandra* were treated with 20, 30, 40, 50 and 60 mM of EMS for 3 and 6 hrs. In the germination test, it was observed that increase in concentration of EMS had adverse effect on seed germination. Similar results have been reported earlier that the mutagenic sensitivity can be attributed to the level of differentiation and development of embryo at the time of treatment and also the extent of damage to the growth components like rate of cell division, cell elongation and various hormones and biosynthetic pathways as observed by Scholz and Lehman (1962). The chemical mutagen EMS revealed that higher reduction of germination and survival occurred at all concentrations of EMS. The observations made in the present study indicated that the reduction in germination and survival in the EMS treatments due to differential age of seeds and the difference in the period between the harvesting of the seeds. It may also be attributed to drop in auxin level (Gordon and Webber, 1955).

In most of the characters, i.e., plant height, internodal length, number of branches, number of leaves, length and breadth of leaf, minimum variances were observed in control plants and the maximum variances were observed in the treated plants. According to Vanthof and sparrow (1963) growth inhibition was a well known response of higher plants to mutagenic treatments which was again attributed in part to the loss of integrity by the proliferous cells in the meristems which give rise to new growth. It is suggested that certain plant tissues may respond differentially to mutagenic treatments depending on the type of growth occurring at the time of treatments. Beheva and patnaik (1975) obtained short mutants in *Amaranthus tricolour* by treating the seeds with EMS solutions. Schiva *et al.* (1984) also obtained decreased plant height in *Gerbera jamesonii* with EMS treatments. The number of leaves is a reflection of nodes. Higher the no. of leaves greater would be the photosynthetic efficiency. Sarafi and Mahdavian (1978) for instance obtained increased number of leaves in carnation by treating the seeds with colchicine. Whereas, no. of branches, length of branch, length and breadth of leaf was supported with similar findings of Beskaravainaya *et al.* (1981) found phenological and morphological variations by treating the seeds of many *Clematis* spp. with ethyl amine, NEU and NMU.

CONLCUSIONS

The results clearly indicate that lower doses of EMS mutagens resulted germination and survival than higher doses. This is the expected result because the control plants are supposed to be genetically similar and any kind of difference observed in the control plants is only due to environment. It is suggest that particular dosage of EMS treatment below the toxic level, can be used to increase the genetic variability in crossandra, which is the basis for any breeding program. Therefore, there is a need to initiate extensive research work using large number of mutagens alone or in combination to induce really desirable variability in vitro and in vivo in order to exploit the same in breeding for developing novel mutants in crossandra which is commercially important loose flower crop.

Table 1: Germination and Survival Per Cent in M_1 Generation

Treatments	Germination Per Cent	Per Cent of Control	Survival Percentage	Per cent of Control	Percentage of Reduction	Seedling Height	Per Cent of Control
Control (wet)	55.50 (48.15)	100.00	40.29 (39.40)	100.00	100.00	13.91	100.00
20	34.76 (36.12)	62.40	37.41 (37.70)	92.85	47.15	12.37	88.92
30	35.64 (36.65)	64.21	20.64 (27.02)	51.22	47.78	12.09	86.91
40	33.49 (35.36)	60.34	21.99 (27.96)	54.57	45.43	10.65	76.56
50	30.43 (33.48)	54.82	20.51 (26.92)	50.90	49.10	9.76	70.16
60	27.76 (31.79)	50.01	20.36 (26.82)	50.53	49.47	8.88	63.83
Mean	**36.26 (37.02)**	**65.33**	**26.86 (31.21)**	**66.66**	**49.82**	**11.27**	**81.06**
SEd	0.64	-	0.56	-	-	0.18	-
CD (P = 0.05)	1.48 *	-	1.25*	-	-	0.38	-

Table 2: Effect of EMS on growth Parameters of Crossandra in M_1 Generation

Treatments	Plant Height	Internodal Length (cm)	No. of Leaves	Length of Leaf (cm)	Breadth of Leaf (cm)	No. of Branches	Length of Branch (cm)
Control (wet)	76.98	3.66	195.43	9.75	4.35	16.81	25.70
EMS 20 mM	84.32	3.74	209.57	10.05	4.67	24.80	25.58
30 mM	82.57	4.36	211.46	10.78	5.26	28.29	29.23
40 mM	74.47	3.54	213.35	10.54	4.45	24.83	26.56
50 mM	69.45	3.45	215.41	9.98	4.34	21.30	22.44
60 mM	65.93	3.39	218.59	9.89	4.26	20.93	20.58
Mean	**75.62**	**3.69**	**210.13**	**10.16**	**4.55**	**22.82**	**25.05**
SEd	1.35	0.06	3.76	0.18	0.08	0.41	0.44
CD (P = 0.05)	3.02**	0.14**	8.39**	0.40**	0.18**	0.91**	1.00**

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